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(54) Title: AN EPITHELIAL PROTEIN AND DNA THEREOF FOR USE IN EARLY CANCER DETECTION

(57) Abstract

The present invention is a purified and isolated epithelial protein, peptide and variants thereof whose increased presence in an epithelial cell is indicative of precancer. One epithelial protein which is an early detection marked for lung cancer was purified from two human lung cancer cell lines, NCI-H720 and NCI-H157. Using a six-step procedure, the epithelial protein was purified using a Western blot detection system under both non-reducing and reducing conditions. Purification steps included anion exchange chromatography, preparative isoelectric focusing, polymer-based C_{18}HPLC and analytic C_4HPLC. After an approximately 25,000 fold purification the immunostaining protein was >90 % pure as judged by coomassie blue staining after reducing SDS-PAGE. The primary epithelial protein share some sequence homology with the heterogeneous nuclear ribonucleoprotein (hnRNP) A2. A minor co-purifying epithelial protein shares some sequence homology with the splice variant hnRNP-B1. Molecular analysis of primary normal bronchial epithelial cell cultures demonstrated a low level the epithelial protein expression, consistent with immunohistochemical staining of clinical samples, and an increased level of expression in most lung cancer cells. The epithelial protein is a marker of epithelial transformation in lung, breast, bone, ovary, prostate, kidney, melanoma and myeloma and may be casual in the process of carcinogenesis. Methods are provided for monitoring the expression of the epithelial protein, peptides and variants using molecular and immunological techniques as a screen for precancer and cancer in mammals. A method of computerized diagnoses of cancer and precancer is provided which detects levels of hnRNP messenger RNA.

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AMENDED CLAIMS

[received by the International Bureau on 21 April 1997 (21.04.97);
new claims 59-67 added; remaining claims unchanged (2 pages)]

54. The method according to claim 53 wherein the optical image is a spatial electronic array.

55. The method according to claim 53 wherein the image is acquired at two different wavelengths.

56. The method according to claim 53 wherein the parameter unique to atypical cells is selected from the group consisting of nuclear texture, nuclear ellipse area, optical density, hnRNP mRNA and combinations thereof.

57. The method according to claim 53 wherein the cell is treated with a labeled probe, said probe specifically hybridized with hnRNP mRNA.

58. The method according to claim 53 wherein the known negative and known atypical cells are from an archived bank of cells taken from normal humans and humans with cancer or precancer.

59. A labeled nucleic acid sequence probe suitable for use in the method according to claim 12.

60. A labeled nucleotide probe according to claim 59 comprising a sequence capable of specifically hybridizing with hnRNP or RNA.

61. The probes of claim 59 or claim 60, wherein said probe is labeled with digoxigenin.

62. The labeled probe of claim 59 or 60 complexed with a nucleic acid sequence complimentary to the labeled probe.

63. The labeled probe of claim 61 employed with a nucleic acid sequence complimentary to the labeled probe.

64. The labeled probe of claim 62 complexed with a nucleic acid sequence complimentary to the labeled probe, and further comprising an antibody complexed to the label on said probe.

65. The labeled probe of claim 64 wherein said antibody is an anti digoxigenin antibody.

66. The nucleic acid sequence probe of claim 12 or claim 43 complexed with a nucleic acid sequence complimentary to said probe.

67. The nucleic acid probes of claims 59, 60 and 66 having the sequence provided in SEQ ID NO:11 or SEQ ID NO:18.